

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68, C07H 21/04		A1	(11) International Publication Number: WO 97/11194 (43) International Publication Date: 27 March 1997 (27.03.97)
(21) International Application Number: PCT/FI96/00501 (22) International Filing Date: 20 September 1996 (20.09.96)		(81) Designated States: AU, CA, CN, JP, NO, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 08/531,070 20 September 1995 (20.09.95) US		Published <i>With international search report.</i>	
(71) Applicant (<i>for all designated States except US</i>): HELSINKI UNIVERSITY LICENSING LTD. OY [FI/FI]; Viikinkaari 8 A, FIN-00710 Helsinki (FI).			
(72) Inventors; and (75) Inventors/Applicants (<i>for US only</i>): DE LA CHAPELLE, Albert [FI/FI]; Vuorimiehenkatu 11, FIN-00140 Helsinki (FI). AIITOMÄKI, Kristiina [FI/FI]; Kuutamokatu 4 B 43, FIN-02210 Espoo (FI). HUHTANIEMI, Ilpo [FI/FI]; Kotimäenkatu 12, FIN-20540 Turku (FI).			
(74) Agent: OY JALO ANT-WUORINEN AB; Iso Roobertinkatu 4-6 A, FIN-00120 Helsinki (FI).			

(54) Title: METHOD FOR DIAGNOSIS OF OVARIAN DYSGENESIS

(57) Abstract

A method is provided for determining a follicle stimulating hormone receptor genotype in a human patient, which method is particularly useful for diagnosing ovarian dysgenesis in affected human females. The method comprises analysis of DNA from a patient which encodes all or a portion of the receptor for follicle-stimulating hormone.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

- 1 -

METHOD FOR DIAGNOSIS OF OVARIAN DYSGENESIS

FIELD OF THE INVENTION

The present invention relates to methods for the detection of hereditary ovarian dysgenesis.

BACKGROUND OF THE INVENTION

Normal gonadal function depends upon the integrity of the pituitary-gonadal axis. In females, regulatory control of the ovary is primarily accomplished by the pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). At birth, development of the ovarian follicles is normally arrested until pituitary gonadotropin stimulation at puberty causes follicular maturation. Either the failure of the pituitary to secrete sufficient amounts of FSH and/or LH or the failure of the ovary to respond to gonadotropin stimulation results in hypogonadism. In females, such a condition may be characterized by the development of anatomically normal internal and external genitalia, variably developed secondary sexual characteristics, and amenorrhea. Failure of the ovaries to respond appropriately to FSH and/or LH stimulation results in poorly-developed (streak) ovaries and increased circulating levels of the hormone(s) to which the ovary is not responding. Such a condition is referred to as ovarian dysgenesis. That disease is one of a group of diseases characterized by hypogonadism which are often referred to generally as hypergonadotropic hypogonadisms. Many such diseases are due to mutations in the sex chromosomes. However, ovarian dysgenesis in females with an XX karyotype is rare and may be due to an autosomal recessive mutation. Simpson, *et al.*, *Birth Defects: Original Article Series*, 7:215-228 (1971); Aittomäki, *Am. J. Genet.*, 54:844-851 (1994).

Also of interest to the present invention are the receptors for FSH and LH. Each of those receptors is a member of a family of receptors which are coupled to GTP-binding proteins (G-proteins) in order to effect intracellular signaling. Other members of this family include certain adrenergic receptors, muscarinic cholinergic receptors, vasopressin receptors, and angiotensin receptors. Most receptors in this family comprise three distinct domains, an extracellular domain, a

- 3 -

thereby generating amplified DNA; exposing the amplified DNA to a restriction endonuclease selected from the group consisting of *BsmI* and restriction endonucleases having a recognition site which overlaps that of *BsmI*, under conditions wherein the restriction endonuclease specifically cleaves DNA at its recognition site; detecting polynucleotide restriction fragments of the amplified DNA; and diagnosing ovarian dysgenesis from the restriction fragments, wherein ovarian dysgenesis is correlated with a C to T mutation in codon 189 of the DNA encoding a receptor for follicle-stimulating hormone, the mutation eliminating a recognition site of the restriction endonuclease. The enzyme *BsmI* recognizes the sequence 5'-
5 NG'CATTC-3' and cleaves between the indicated G and C. Restriction endonucleases that recognize sequences which overlap the recognition site of *BsmI*, such as *SacIII* or *SstIII*, may also be used. *SacIII* and *SstIII* recognize sequences internal to a *BsmI* site.

The portion of nucleic acids subjected to amplification need not include the entire coding sequence for a follicle-stimulating hormone receptor. For example, the portion of nucleic acids amplified in the method of the invention may include only exon 7, or only a portion of exon 7. Depending upon the particular portion of nucleic acid amplified in the method of the invention, and the particular restriction enzyme used, the number and/or size of restriction fragments may vary.
For example, using nucleic acids comprising exon 7 of a follicle-stimulating hormone receptor (*fshr*) allele and *BsmI*, a diagnosis of ovarian dysgenesis is made if the amplified nucleic acid remained uncut when exposed to the restriction endonuclease (*i.e.*, a single fragment comprising the uncut amplified nucleic acid is observed).

In another preferred embodiment, the invention provides a method for diagnosing ovarian dysgenesis in a female patient suspected of having ovarian dysgenesis, comprising the steps of obtaining a cell sample from the female patient; isolating nucleic acid from the cell sample; amplifying a portion of the isolated nucleic acid comprising exon 7 of a follicle-stimulating hormone receptor gene, thereby generating amplified DNA; sequencing the amplified DNA; comparing the sequence of the amplified DNA to SEQ ID NO:1; and diagnosing ovarian dysgenesis by the presence of one or more sequence differences between the sequence

- 4 -

of the amplified DNA and SEQ ID NO:1. The sequence differences, for example, may involve a single nucleotide difference at position 640 of SEQ ID NO:1 (e.g., a thymidylate in the amplified DNA corresponding to the cytidylate at position 640 of SEQ ID NO:1).

5 Another aspect of the invention is directed to a method for determining a follicle-stimulating hormone receptor (*fshr*) genotype in a human patient, which comprises the steps of providing a biological sample comprising nucleic acid from the patient, the nucleic acid including the patient's *fshr* alleles; analyzing the nucleic acid for the presence of a mutation or mutations in codon 189 of the *fshr* alleles; and determining an *fshr* genotype from the analyzing step, wherein the presence of a mutation in codon 189 of a *fshr* allele is correlated with an ovarian dysgenesis genotype. The method is useful for screening both male and female patients for determining an *fshr* genotype, for predicting/diagnosing disease states (e.g., ovarian dysgenesis in a female patient), and for genetic counseling purposes
10 (e.g., for couples intending to have children). The biological sample may be any sample from the patient containing nucleic acid suitable for analysis (e.g., tissue and fluid samples); a cell sample is a preferred biological sample. Further, the analyzing step of the invention embraces a variety of analytical techniques including sequencing all, or a portion, of a nucleic acid (which may be DNA or RNA) comprising codon 189 of a *fshr* allele. By way of further illustration, the analyzing step of the invention includes restriction endonuclease analyses, wherein a nucleic acid is exposed to a restriction endonuclease having a recognition site that includes codon 189 of a wild type *fshr* allele, under conditions wherein the restriction endonuclease specifically cleaves DNA at its recognition site; and detecting
15 the nucleic acid or polynucleotide restriction fragments thereof resulting from the exposing step. Restriction endonucleases for use in this embodiment of the invention include *Bsm*I, restriction endonucleases recognizing sites that overlap a *Bsm*I site, restriction endonucleases recognizing the same cleavage sites as *Bsm*I, and isoschizomers of *Bsm*I.
20
25

30 In another aspect of the invention, a method for screening for an ovarian dysgenesis genotype in a patient comprises the steps of providing a biolo-

- 5 -

gical sample comprising nucleic acid from the patient, the nucleic acid including the patient's follicle-stimulating hormone receptor (*fshr*) alleles; amplifying a portion of the nucleic acid to generate amplified DNA, the portion including codon 189 of the *fshr* alleles; exposing the amplified DNA to a restriction endonuclease selected from the group consisting of *Bsm*I restriction endonuclease and restriction endonucleases having a recognition site which overlaps a *Bsm*I recognition site, under conditions wherein the restriction endonuclease specifically cleaves DNA at the recognition site of the restriction endonuclease; thereafter detecting said amplified DNA or restriction fragments thereof; comparing the amplified DNA or fragments thereof to control nucleic acid of a human subject free of an ovarian dysgenesis genotype, wherein said control nucleic acid has been amplified, exposed, and detected as described above; and screening for an ovarian dysgenesis from the comparison, wherein an ovarian dysgenesis genotype in the patient is correlated with a different number of detected amplified DNA or restriction fragments thereof from the patient's amplified DNA than from the control amplified DNA. In screening for ovarian dysgenesis genotypes, for example, a homozygous ovarian dysgenesis genotype may be correlated with fewer detected restriction fragments from the patient's amplified DNA than from the control amplified DNA. Consistently, the screening may correlate a heterozygous ovarian dysgenesis genotype with a greater number of detected amplified DNA or restriction fragments thereof from the patient's amplified DNA than from the control DNA.

Yet another aspect of the invention is a method comprising the steps of obtaining a cell sample from a female patient; isolating DNA from the cell sample; amplifying a portion of the DNA encoding exon 7 of the follicle-stimulating hormone receptor; exposing the amplified DNA to *Bsm*I or another restriction endonuclease which recognizes the *Bsm*I site; and diagnosing ovarian dysgenesis as the presence of a single fragment upon digestion.

Additional aspects of the invention will become apparent upon consideration of the following detailed description thereof.

- 6 -

DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of the human FSH receptor showing the multi-exon structure of the gene encoding the protein; the extracellular (Ec), transmembrane (Tm), and intracellular (Ic) domains of the receptor; and the 5 Ala to Val and Asn to Ser transitions (boxed) described herein.

Figure 2 shows a pedigree of families used in studies of ovarian dysgenesis inheritance patterns.

Figure 3 is a map of microsatellite markers on human chromosome 2.

10 Figure 4 depicts the nucleotide sequence, and deduced amino acid sequence, of the FSH receptor (SEQ ID NO:1) gene, showing *Bsm*I sites in bold.

Figure 5 is a graph showing results of FSH-induced cAMP production by MSC01 cells transfected with wild-type or mutant FSH receptor-encoding DNA.

15 Figure 6 is a graph showing Scatchard analysis of FSH binding to MSC-1 cells transfected with wild-type (circles) or mutated (triangles) FSH receptor-encoding DNA.

DETAILED DESCRIPTION OF THE INVENTION

20 The present invention provides methods for the diagnosis of diseases characterized by hypergonadotropic hypogonadisms such as ovarian dysfunction. In particular, methods of the invention are useful in the diagnosis of ovarian dysgenesis in females. Diagnostic methods according to the invention may be used at any time in the life of the patient, even prior to the onset of symptoms, such as the 25 failure of normal onset of menstruation at puberty. Methods of the invention are primarily based upon the discovery that a mutation in the coding sequence of the gene for the follicle-stimulating hormone receptor accounts for the inability of FSH to generate signal transduction at its receptor.

In the normal genotype for the follicle-stimulating hormone receptor, 30 four *Bsm*I restriction sites (5'-NGCATTG-3') exist, as shown in Figure 4 (SEQ ID NO:1). In individuals having ovarian dysgenesis, the FSH receptor gene has been observed to have a mutation at position 640 (SEQ ID NO:1), such that a thymine

- 7 -

is substituted for a cytosine, resulting in the sequence, 5'-NGTATTC-3'. This substitution eliminates the *BsmI* site spanning nucleotides 638-644 in the normal (wild type) fshr gene. Thus, digestion of DNA encoding a wild-type FSH receptor with *BsmI* normally produces five fragments (due to cleavage at all four *BsmI* sites in a linear DNA). However, the thymine-to-cytosine mutation at position 640 results in a mutated DNA that produces only four restriction fragments upon *BsmI* digestion. As shown below, mutation at position 640 is uniquely associated with ovarian dysgenesis; whereas no mutation at any of the other three *BsmI* sites in the FSH receptor DNA has been found in patients suffering from that disease. Accordingly, *BsmI* digestion and subsequent observation of the products produced is useful as a diagnostic method or a screening method for ovarian dysgenesis. As detailed below, position 640 in SEQ ID NO:1 falls within exon 7 of the fshr gene. In one embodiment of the invention, the region of exon 7, or a portion thereof, of the FSH receptor coding sequence may also be isolated and exposed to *BsmI*. Exon 7 contains a unique *BsmI* site which, if mutated, will produce no *BsmI* digestion products, thereby enabling diagnosis of ovarian dysgenesis.

The gene encoding the FSH receptor has been mapped to human chromosome 2p16-21. Rousseau-Merck, *et al.*, *Genomics*, 15:222-224 (1993). The FSH receptor coding sequence is shown in SEQ ID NO:1 and in Figure 4 and is available in the Genbank database as Accession No. S59900. Figure 1 shows a schematic of the FSH receptor showing the regions encoded by the 10 exons.

The following examples illustrate preferred means for detecting FSH receptor genotypes generally and for diagnosis of ovarian dysgenesis in human females.

25

EXAMPLE 1

Venous blood samples were obtained from 37 individuals, each belonging to one of 6 families having at least two females with symptoms of ovarian dysgenesis. Total DNA was isolated from fresh white blood cells or lymphoblastoid cell lines established from the samples. The method of Chomczynski, *et al.*, *Anal. Biochem.*, 162:156-159 (1987), incorporated by reference herein, was

- 8 -

used to isolate RNA from the samples. A pedigree showing inheritance patterns in individuals used to isolate the ovarian dysgenesis locus is shown in Figure 2.

Linkage was investigated using Généthon microsatellite markers as reported in Weissenbach, *et al.*, *Nature*, 359:794-801 (1992), incorporated by

5 reference herein. A linkage map for chromosome 2 is provided in Figure 3.

Forty-seven markers were chosen at approximately 20 centimorgan (cM) intervals.

Pairwise linkage analyses were carried out using the MLINK subprogram of the

LINKAGE program package reported in Lathrop, *et al.*, *Proc. Nat'l. Acad. Sci. (USA)*, 81:3443-3446 (1984), incorporated herein by reference. The disease allele

10 frequency was set to 0.01 and lod scores were computed under a model of equilibrium between the disease locus and each marker locus, assuming a recessive mode of inheritance with full penetrance in females. A lod score is defined as the

log₁₀ of the ratio of the probability that the data obtained would have arisen from unlinked loci and represents a criterion for assigning a given restriction fragment

15 length polymorphism to a particular linkage group. Linkage was confirmed using the G2113A transition located in exon 10 of the FSH receptor gene as a polymorphic marker (see below). Amplification was accomplished using PCR according to the method of Sankila, *et al.*, *Hum. Mol. Genet.*, 4:93-98 (1995), incorporated by reference herein.

20 Preliminary evidence of linkage of the ovarian dysgenesis phenotype was noted for markers D2S134 and D2S177 on chromosome 2. Linkage was confirmed using markers D2S119 and D2S123, each of which is located between D2S134 and D2S177. Markers D2S119 and D2S123 define an approximately 12 cM region of chromosome 2. That region was further studied using markers

25 D2S391, D2S288, CA21 and CA7, each of which is located in the 12 cM region defined above, and linkage of the ovarian dysgenesis locus to this region of chromosome 2 was confirmed.

30 Pairwise linkage analyses produced 46 potentially informative meioses, of which 30 were from affected females. The lod scores obtained from those meioses revealed no recombinations between the markers and the ovarian dysgenesis locus as evidenced by phenotype. The highest lod score, $Z_{\text{max}} = 4.71$, was obtained for marker D2S391, while two other marker loci, D2S288 and CA21,

- 9 -

provided lod scores exceeding the limit of proven linkage ($Z \geq 3.0$). The region in which linkage of the ovarian dysgenesis phenotype exists was, therefore, isolated to the 2p region of chromosome 2 based upon the above linkage analysis. That region of chromosome 2 is the region which contains the genes encoding the receptors for follicle-stimulating hormone and luteinizing hormone. Thus, it was hypothesized that a nonlethal mutation in one of these two genes was responsible for ovarian dysgenesis. On the basis that an LH receptor mutation would also cause severe hypogonadism in males and no males were affected in the pedigrees producing affected females, the LH receptor gene was considered unlikely as a site of mutation causing ovarian dysgenesis. Accordingly, the gene encoding the FSH receptor was investigated as a source of the ovarian dysgenesis phenotype.

EXAMPLE 2

As shown in Figure 1, the FSH receptor gene comprises 10 exons.

15 As recited above, the polynucleotide sequence of the entire *fshr* coding region can be obtained from Genbank under Accession Number S59900. The polynucleotide sequence of each one of the ten exons of *fshr* has also been deposited under individual accession numbers in the Genbank database. A comparison of the sequences of the individual exons to the coding region of the entire coding region presented in SEQ ID NO:1 reveals that a few terminal nucleotides from each exon are lost in the process of forming a mature mRNA during expression. The correlation between the sequences of the individual exons and the sequence presented in SEQ ID NO:1 follows. Nucleotides 1-158 of exon 1 (Acc. No. X91738) are found at positions 70-227 of SEQ ID NO:1; nucleotides 7-77 of exon 2 (Acc. No. X91739) span positions 228-298 of SEQ ID NO:1; nucleotides 6-80 of exon 3 (Acc. No. X91740) are found at positions 299-373 of SEQ ID NO:1; nucleotides 6-82 of exon 4 (Acc. No. X91741) span positions 374-450 of SEQ ID NO:1; nucleotides 8-77 of exon 5 (Acc. No. X91742) are found at positions 451-520 of SEQ ID NO:1; nucleotides 6-83 of exon 6 (Acc. No. X91743) span positions 521-598 of SEQ ID NO:1; nucleotides 6-75 of exon 7 (Acc. No. X91744) are found at positions 599-668 of SEQ ID NO:1; nucleotides 7-80 of exon 8 (Acc. No. X91745) span positions 669-742 of SEQ ID NO:1; nucleotides 6-191 of exon 9 (Acc. No.

- 10 -

X91746) are found at positions 743-928 of SEQ ID NO:1; and nucleotides 102-1352 of exon 10 (Acc. No. S73526) span positions 929-2179 of SEQ ID NO:1.

A systematic analysis was performed to determine the change or changes in that gene which are responsible for expression of the ovarian dysgenesis phenotype.

The large terminal exon of the FSH receptor was screened for mutations using denaturing gradient gel electrophoresis. Exon 10 was amplified with GC clamped primers in four different PCR reactions with overlapping products covering the entire transmembrane and intracellular domains. Pairs of GC clamped primers used in PCR were primer 10f: 5'-CGCCCGCCGCGCCCCGCC-
10 GGCCCGCCGCCCGCCGGACTTATGCAATGAAGTGGTTG-3' (forward, SEQ ID NO:2), 10r: 5'-GTGAAAAAGCCAGCAGCATC-3' (reverse, SEQ ID NO:3); primer 11f: 5'-CGCCCGCCGCGCCCCGCCGGCCGCCGCCGCC-
15 GCCCGATTGACTGGCAAACGGGG-3' (forward, SEQ ID NO:4), 11r: 5'-AGAGGAGGACACGATGTTGG-3' (reverse, SEQ ID NO:5); primer 12f: 5'-CGCCCGCCGCGCCCCGCCGGCCGCCGCCGCCGGCTGCTA-
15 TATCCACATCTACC-3' (forward, SEQ ID NO:6), 12r: 5'-CAGAACCAAGCA-
20 GAATCTTG-3' (reverse, SEQ ID NO:7); and primer 13f: 5'-CGCCCGCCGCGCCCCGCCGGCCGCCGCCGCCGCTTCTTG-
25 CATTCTGCC-3' (forward, SEQ ID NO:8), 13r 5'-CAAAGGCAAGGACT-
GAATTATC-3' (reverse, SEQ ID NO:9). Each pair of primers was optimized for each fragment with the MELT 87 program described in Lerman, *et al.*, *Methods in Enzymology* (Wu, *et al.*, eds. 1989), incorporated by reference herein. For each PCR run, samples were heated to 94°C for 4 minutes and put through 34 cycles.
25 For each cycle, denaturation was conducted for 1 minute at 94°C, annealing was conducted for 1 minute at 57°C, 58°C, 57°C, and 54°C for primer pairs 10, 11, 12, and 13, respectively, and extension was for 30 seconds at 72°C.

A 7% polyacrylamide gel was designed for each fragment with a linear concentration gradient of formamide. Gels were run in an aquarium at a stable temperature of about 60°C for 16-20 hours in a denaturing gel electrophoresis system, model DGGE2000 (C.B.S. Scientific Co.). Denaturing gradient gel electrophoresis procedures are generally known in the art and are available, for

- 11 -

example, in Ausubel, *et al.* (eds.) *Current Protocols in Molecular Biology*, 2.12, *et seq.* (1987), incorporated by reference herein.

A sequence polymorphism was detected in the terminal region of exon 10. A 326 bp fragment beginning at nucleotide 1892 of the FSH receptor coding sequence was amplified using primers 14f: 5'-AGCAAAGATTCTGCTGG-TTC-3' (forward, SEQ ID NO:10) and 14r: 5'-CAAAGGCAAGGACTGAAT-TATC-3' (reverse, SEQ ID NO:9). The amplified PCR product was sequenced using the dideoxy chain termination method as reported in *Molecular Cloning: A Laboratory Manual* (Sambrook, *et al.*, eds 1989), incorporated by reference herein. A G-to-A transition was observed in the sequenced product at nucleotide position 2113 (G2113A transition). Position 2113 of SEQ ID NO:1 is in the region encoding the intracellular domain of the FSH receptor and predicts a change from Ser to Asn at amino acid position 680 of the corresponding protein structure. The G2113A transition abolishes a *Bsrl* site, thus enabling *Bsrl* to be used as a screen for the two alleles. The polymorphism at that site was designated FSHR1.

The G2113A transition observed in exon 10 of the FSH receptor coding sequence had previously been identified as a difference between the ovarian and testicular forms of the gene encoding the FSH receptor. Kelton, *et al.*, *Mol. Cell. Endocrinol.*, 89:141-151 (1992). Denaturing gradient gel electrophoresis analysis of samples obtained from a family having females both with and without ovarian dysgenesis showed that the allelic form of the gene at the G2113A transition had no affect on phenotype. Thus, the allelic variance at position 2113 was not considered to be disease causing. No recombinations between that locus and the disease phenotype were observed, as shown in Table 1.

- 12 -

TABLE 1

Recombination fraction, Θ									
	Marker locus	0.00	0.001	0.01	0.05	0.10	0.20	0.30	90% support interval
5	<i>D2S119</i>	2.41	2.41	2.35	2.12	1.82	1.20	0.62	0-0.17
	<i>D2S391</i>	4.71	4.70	4.59	4.14	3.56	2.38	1.26	0-0.09
	<i>D2S288</i>	3.10	3.10	3.03	2.72	2.33	1.52	0.78	0-0.13
	<i>CA21</i>	4.20	4.19	4.11	3.74	3.24	2.19	1.16	0-0.10
	<i>CA7</i>	2.66	2.66	2.60	2.35	2.03	1.36	0.71	0-0.16
10	<i>D2S123</i>	2.68	2.67	2.62	2.39	2.08	1.44	0.79	0-0.16
	<i>FSHR1</i>	2.72	2.71	2.66	2.44	2.14	1.49	0.82	0-0.16

15 The FSHR1 locus was, however, used as a marker in the linkage analysis described above. Since the polymorphism in exon 10 was excluded as the source of the ovarian dysgenesis phenotype, the other exons of the FSH receptor were screened.

20

EXAMPLE 3

Exons 1-5 and 9 were screened by amplifying each with flanking intronic primers and sequencing the PCR product. None of those exons showed a mutation associated with the ovarian dysgenesis phenotype.

25 Complete sequences for the flanking introns of exons 6, 7, and 8 were not available. However, it was determined that FSH receptor-encoding mRNA could be isolated by using RNA from blood leukocytes as a template. Such a process takes advantage of so-called illegitimate transcription, whereby small amounts of mRNA encoding most tissue-specific proteins are produced by white blood cells. Due to the sensitivity of PCR and since PCR products can be directly sequenced, small amounts of RNA produced by white blood cells were used to amplify the FSH receptor coding region. RNA was isolated from white

- 13 -

blood cells and exons 6-9 were amplified by reverse transcription PCR in order to identify mutations by direct sequencing of the PCR products as follows.

Total RNA was isolated from white blood cells or from lymphoblasts. Approximately 0.8 µg RNA was used as a template for first-strand cDNA synthesis and primed for reverse transcription by 40 pmol of primer 15r: 5'-
5 TAGTTTGGGCTAAATGACTTAGAGGG-3' (SEQ ID NO:11), which is complementary to nucleotides 2161-2135 of SEQ ID NO:1. Approximately 1mM each of dATP, dCTP, dTTP, dGTP and 200 U M-mLv reverse transcriptase were combined in M-mLv reverse transcriptase buffer (Promega, Madison, WI) to a final volume 20 µL. Samples were incubated at 42°C for 1 hour. Samples were then heated to 95°C for 10 minutes and a 5 µl aliquot of the resulting cDNA product was used as a template for PCR.

The cDNA was amplified in two rounds of PCR using a nesting strategy in order to increase yield and specificity. In the first round of PCR, 15 primer 15r (see above) and primer 16f: 5'-CCTGCTCCTGGTCTCTTGCTG-3' (SEQ ID NO:12) were used. The reaction was heated for 2 minutes at 94°C and put through 20 cycles each comprising 1 minute denaturing at 94°C, 1 minute annealing at 58°C and 2 minutes extension at 73°C to produce a 2082 bp product. The product contained exons 6-9 and 5µl was used as a template for amplification 20 of exon 7 using primer 6f: 5'-AGAAATTCTTCGTGGGCT-3' (forward, SEQ ID NO:13) and 6r: 5'-GTTTGCAAAGGCACAGCAAT-3' (reverse, SEQ ID NO:14). The resulting PCR product was a 357 bp fragment corresponding to nucleotides 558-914 of SEQ ID NO:1.

The 357 bp product, which included exon 7, was sequenced. Upon 25 inspection of the resulting sequence in a number of patients (both affected and unaffected), it was noticed that all affected individuals were homozygous for a C-to-T transition at nucleotide 640 (C640T transition) of the FSH receptor sequence shown in SEQ ID NO:1. That change predicts an Ala-to-Val substitution at amino acid position 189 in the corresponding protein primary structure. The C640T 30 transition segregated perfectly with the disease phenotype and all affected individuals were determined to be homozygous recessive for the mutation from cysteine to thymine. The mutation at position 640 abolishes a *Bsm*I restriction site, thus

- 14 -

enabling diagnosis of the disease by digestion of FSH receptor DNA with *BsmI*. Thus, it was determined that the C640T transition in exon 7 of the FSH receptor gene is predictive of ovarian dysgenesis in affected individuals.

5

EXAMPLE 4

Digestion of genomic DNA with *BsmI* was next used in order to confirm the use of that enzyme in the diagnosis of ovarian dysgenesis.

Genomic DNA isolated from 15 affected and 22 unaffected individuals was amplified by PCR by heating for 12 minutes at 94°C followed by 30 cycles 10 at 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes using primers 7f: 5'-GTTATTCAGATGGCTGAATAAG-3' (SEQ ID NO:15) and 7r: 5'-GCTCATCTAGTTGGGTTC-3' (SEQ ID NO:16). These primers were selected to amplify a 78 base pair portion of genomic DNA encompassing a portion of the intron between exons 6 and 7 and most of exon 7 of the follicle stimulating hormone receptor gene. Exon 7 of the wild type *fshr* gene contains a unique *BsmI* site at nucleotides 638-644 of SEQ ID NO: 1 which is abolished by the C640T transition observed in individuals with the ovarian dysgenesis phenotype (the three additional *BsmI* sites in the FSH receptor coding sequence shown in Fig. 4 are not in exon 7 and are not included in the above-described PCR product). Fifteen 15 microliters of the resulting 78 bp PCR product was digested to completion with 20 IU of *BsmI* (Promega). The product of digestion was fractionated on a 10% non-denaturing polyacrylamide gel and the DNA was visualized with ethidium bromide. 20

In unaffected individuals, the PCR product is cleaved into 51 bp and 27 bp fragments by *BsmI*. However, in affected individuals, only a 78 bp fragment appears in the gel. Heterozygous individuals, such as the parents shown in 25 the Family 7 pedigree in Figure 2, show all three fragments. Thus, *BsmI* digestion is probative of ovarian dysgenesis by detecting the C640T transition. The pathogenic role of the C640T transition was further confirmed in FSH receptor signaling and binding studies.

30

- 15 -

EXAMPLE 5

Human FSH receptor-encoding DNA was obtained by reverse transcription PCR using testicular poly (A⁺) RNA as reported in Gromoll, *et al.*, *Biochem. Biophys. Res. Commun.*, 188:1077-1083 (1992), incorporated by reference herein. The resulting PCR product was subcloned into a pBluescript SR(-) vector (Stratagene Inc., La Jolla, CA). A 5' untranslated region which contained a stop codon was deleted and a Kozak translation initiation sequence was added. The resulting construct comprised 2088 bp of the FSH receptor coding region with a 5-base 5' extension and a 92-base 3' extension. The FSH receptor coding sequence with 5' and 3' extensions was excised by digestion with *Sma*I and *Kpn*I and blunt-end ligated into a blunted *Eco*RI site in the pSG5 vector (Stratagene).

A plasmid comprising the mutated receptor (*i.e.* the C640T allele) was created by site-directed mutagenesis of the wild-type cDNA. Site-directed mutagenesis was accomplished using a Clontech Transformer™ site-directed mutagenesis kit (Clontech, Palo Alto, CA) according to the manufacturer's instructions. The selection primer, 5'-GAGTGCACCATGGCGGTGTGAAAT-3' (SEQ ID NO:17), transformed an *Nde*I site into an *Nco*I site in the vector. The mutagenesis primer, 5'-GGGATTCAAGAAATACACAACGTGTATTCAATGGAACCC-3' (SEQ ID NO:18), accomplishes the C-to-T transition at position 640. Plasmid sequences were verified by restriction digestion and sequencing.

An MSC-1 cell line was used for transfection. That cell line was derived from a transgenic mouse Sertoli cell tumor generated by expressing the SV40 virus T-antigen under the control of the anti-muellerian hormone promoter reported in Peschon, *et al.*, *Mol. Endocrinol.*, 6:1403-1411 (1992), incorporated by reference herein. Despite their Sertoli cell origin, MSC-1 cells do not express endogenous FSH receptor. MSC-1 cells in exponential growth phase were transiently transfected with either the wild-type or mutated FSH receptor-containing plasmids described above. Transfection was accomplished in DMEM/F12 (1:1) medium using lipofection (Gibco Life Technologies, Inc.) according to the manufacturer's instruction. A mock transfection with buffer was run as a control. Transfection efficiency was maintained by cotransfection with a luciferase-expressing pCmv-luci plasmid as reported in Gossen, *et al.*, *Proc. Nat'l. Acad. Sci.*

- 16 -

(USA), 89:5547-5551 (1992). Transfected cells were cultured in 2 cm culture plates.

Seventy-two hours after transfection, cells were exposed to one of 2, 10, 50, 100, or 200 IU/L of recombinant human FSH (rhFSH, Org 32489, approximately 10,000 IU/mg, Organon International BV). A control (vehicle only) was also run. After 3 hours, cells and media were removed from the culture plates and divided into two equal aliquots. One of the aliquots was diluted 1:1 with 2 mM theophylline, heated for 5 minutes at 100°C, spun for 5 minutes at 1500 g; and used to measure cAMP activity as described in Harper, *et al.*, *J. Cycl. Nucleotide Res.*, 1:207-218 (1975), incorporated by reference herein.

Stimulation with rhFSH of MSC-1 cells transfected with wild-type FSH receptor DNA produced a 3-4 fold dose-dependent stimulation of cAMP. The ED₅₀ of stimulation was approximately 75 IU/L. In contrast, cells transfected with DNA comprising the C640T allele or mock-transfected with only pCmv-luci produced only negligible increases in cAMP activity, indicating that such cells are not stimulated by FSH. The results are shown in Figure 5, wherein squares denote transfections with the wild type allele, circles denote transfections with the mutant allele, and triangles represent mock transfections. Each data point in Figure 5 represents the mean of results in three identical experiments.

The foregoing results show that the ovarian dysgenesis allele (*i.e.* that with a substitution of T for C at position 640) results in the expression of a receptor which is unable to produce a signal upon stimulation by FSH.

EXAMPLE 6

MSC-1 cells which had been transfected with either mutant or wild-type FSH receptor plasmids were next used in FSH binding studies performed 48 hours after transfection. Cells were recovered and reconstituted to a concentration of 2x10⁶ cells/ml in buffer. The rhFSH described above was radiolabelled with ¹²⁵I iodine using the solid-phase lactoperoxidase method of Karonen, *et al.*, *Anal. Biochem.*, 67:1-10 (1975), incorporated by reference herein, to a specific activity of 30 Ci/g and 20% specific binding of radioactivity to an excess of FSH receptor as determined according to Catt, *et al.*, *Methods in Receptor Research* (Belcher,

- 17 -

ed. 1976) 175-250, incorporated by reference herein. Triplicate aliquots of 100 μ l each of the cell suspension (containing approximately 200,000 cells each) were incubated in the presence of 3.13, 6.25, 12.5, 25, 50, or 100 ng of radiolabelled rhFSH in a total volume of 250 μ l. Non-specific binding was determined in the 5 presence of 1.5 IU rhFSH. After overnight incubation at room temperature, radioactivity was measured in a gamma spectrometer.

Scatchard analysis revealed a 28-fold increase in binding of FSH to cells which had been transfected with wild-type FSH receptor-encoding DNA compared to cells transfected with DNA encoding the C640T allele. The equilibrium constant of FSH binding was $K_a = 6.7 \times 10^9$ L/mol for the wild-type receptor 10 and 4.8×10^9 L/mol for the mutated receptor. Results are shown in Figure 6, wherein circles represent wild-type receptors and triangles represent mutant receptors. In each case specific binding was equalized to a constant amount of luciferase activity. As shown in Figure 6, specific binding was 18.2 pmol/L for the wild 15 type and 0.63 pmol/L for the ovarian dysgenesis allele.

The foregoing results show that a mutation from C to T at position 640 of the FSH receptor coding sequence is responsible for ovarian dysgenesis in human females and that because that mutation abolishes a *BsmI* site, digestion with *BsmI* or another restriction endonuclease which recognizes the *BsmI* site is useful 20 as a diagnostic tool for ovarian dysgenesis. It is apparent from the foregoing that other mutations may also produce the ovarian dysgenesis phenotype. Comparison of the nucleotide sequence of the FSH receptor gene in those cases with the wild-type sequence also provides a basis for diagnosis of the disease.

- 18 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Helsinki University Licensing Ltd Oy
- (B) STREET: Viikinkaari 8 A
- (C) CITY: Helsinki
- (E) COUNTRY: Finland
- (F) POSTAL CODE: FIN-00710

(ii) TITLE OF INVENTION: Method For Diagnosis Of Ovarian Dysgenesis

(iii) NUMBER OF SEQUENCES: 18

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Oy Jalo Ant-Wuorinen Ab
- (B) STREET: Iso Roobertinkatu 4-6 A
- (C) CITY: Helsinki
- (E) COUNTRY: Finland
- (F) POSTAL CODE: FIN-00120

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 08/531,070
- (B) FILING DATE: 20-SEP-1995

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Karvinen, Leena
- (C) REFERENCE/DOCKET NUMBER: 29160

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 358 0 648606
- (B) TELEFAX: 358 0 640 575
- (C) TELEX: 123505 JALO SF

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2179 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 75..2159

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTGGAGCTT CTGAGATCTG TGGAGGTTTT TCTCTGCAAA TGCAGGAAGA AATCAGGTGG

- 19 -

ATGGATGCAT AATT ATG GCC CTG CTC CTG GTC TCT TTG CTG GCA TTC CTG Met Ala Leu Leu Leu Val Ser Leu Leu Ala Phe Leu 1 5 10	110
AGC TTG GGC TCA GGA TGT CAT CAT CGG ATC TGT CAC TGC TCT AAC AGG Ser Leu Gly Ser Gly Cys His His Arg Ile Cys His Cys Ser Asn Arg 15 20 25	158
GTT TTT CTC TGC CAA GAG AGC AAG GTG ACA GAG ATT CCT TCT GAC CTC Val Phe Leu Cys Gln Glu Ser Lys Val Thr Glu Ile Pro Ser Asp Leu 30 35 40	206
CCG AGG AAT GCC ATT GAA CTG AGG TTT GTC CTC ACC AAG CTT CGA GTC Pro Arg Asn Ala Ile Glu Leu Arg Phe Val Leu Thr Lys Leu Arg Val 45 50 55 60	254
ATC CAA AAA GGT GCA TTT TCA GGA TTT GGG GAC CTG GAG AAA ATA GAG Ile Gln Lys Gly Ala Phe Ser Gly Phe Gly Asp Leu Glu Lys Ile Glu 65 70 75	302
ATC TCT CAG AAT GAT GTC TTG GAG GTG ATA GAG GCA GAT GTG TTC TCC Ile Ser Gln Asn Asp Val Leu Glu Val Ile Glu Ala Asp Val Phe Ser 80 85 90	350
AAC CTT CCC AAA TTA CAT GAA ATT AGA ATT GAA AAG GCC AAC AAC CTG Asn Leu Pro Lys Leu His Glu Ile Arg Ile Glu Lys Ala Asn Asn Leu 95 100 105	398
CTC TAC ATC AAC CCT GAG GCC TTC CAG AAC CTT CCC AAC CTT CAA TAT Leu Tyr Ile Asn Pro Glu Ala Phe Gln Asn Leu Pro Asn Leu Gln Tyr 110 115 120	446
CTG TTA ATA TCC AAC ACA GGT ATT AAG CAC CTT CCA GAT GTT CAC AAG Leu Leu Ile Ser Asn Thr Gly Ile Lys His Leu Pro Asp Val His Lys 125 130 135 140	494
ATT CAT TCT CTC CAA AAA GTT TTA CTT GAC ATT CAA GAT AAC ATA AAC Ile His Ser Leu Gln Lys Val Leu Leu Asp Ile Gln Asp Asn Ile Asn 145 150 155	542
ATC CAC ACA ATT GAA AGA AAT TCT TTC GTG GGG CTG AGC TTT GAA AGT Ile His Thr Ile Glu Arg Asn Ser Phe Val Gly Leu Ser Phe Glu Ser 160 165 170	590
GTG ATT CTA TGG CTG AAT AAG AAT GGG ATT CAA GAA ATA CAC AAC TGT Val Ile Leu Trp Leu Asn Lys Asn Gly Ile Gln Glu Ile His Asn Cys 175 180 185	638
GCA TTC AAT GGA ACC CAA CTA GAT GAG CTG AAT CTA AGC GAT AAT AAT Ala Phe Asn Gly Thr Gln Leu Asp Glu Leu Asn Leu Ser Asp Asn Asn 190 195 200	686
AAT TTA GAA GAA TTG CCT AAT GAT GTT TTC CAC GGA GCC TCT GGA CCA Asn Leu Glu Glu Leu Pro Asn Asp Val Phe His Gly Ala Ser Gly Pro 205 210 215 220	734
GTC ATT CTA GAT ATT TCA AGA ACA AGG ATC CAT TCC CTG CCT AGC TAT Val Ile Leu Asp Ile Ser Arg Thr Arg Ile His Ser Leu Pro Ser Tyr 225 230 235	782
GGC TTA GAA AAT CTT AAG AAG CTG AGG GCC AGG TCG ACT TAC AAC TTA Gly Leu Glu Asn Leu Lys Lys Leu Arg Ala Arg Ser Thr Tyr Asn Leu 240 245 250	830

- 20 -

AAA AAG CTG CCT ACT CTG GAA AAG CTT GTC GCC CTC ATG GAA GCC AGC Lys Lys Leu Pro Thr Leu Glu Lys Leu Val Ala Leu Met Glu Ala Ser 255 260 265	878
CTC ACC TAT CCC AGC CAT TGC TGT GCC TTT GCA AAC TGG AGA CGG CAA Leu Thr Tyr Pro Ser His Cys Cys Ala Phe Ala Asn Trp Arg Arg Gln 270 275 280	926
ATC TCT GAG CTT CAT CCA ATT TGC AAC AAA TCT ATT TTA AGG CAA GAA Ile Ser Glu Leu His Pro Ile Cys Asn Lys Ser Ile Leu Arg Gln Glu 285 290 295 300	974
GTT GAT TAT ATG ACT CAG ACT AGG GGT CAG AGA TCC TCT CTG GCA GAA Val Asp Tyr Met Thr Arg Gly Gln Arg Ser Ser Leu Ala Glu 305 310 315	1022
GAC AAT GAG TCC AGC TAC AGC AGA GGA TTT GAC ATG ACG TAC ACT GAG Asp Asn Glu Ser Ser Tyr Ser Arg Gly Phe Asp Met Thr Tyr Thr Glu 320 325 330	1070
TTT GAC TAT GAC TTA TGC AAT GAA GTG GTT GAC GTG ACC TGC TCC CCT Phe Asp Tyr Asp Leu Cys Asn Glu Val Val Asp Val Thr Cys Ser Pro 335 340 345	1118
AAG CCA GAT GCA TTC AAC CCA TGT GAA GAT ATC ATG GGG TAC AAC ATC Lys Pro Asp Ala Phe Asn Pro Cys Glu Asp Ile Met Gly Tyr Asn Ile 350 355 360	1166
CTC AGA GTC CTG ATA TGG TTT ATC AGC ATC CTG GCC ATC ACT GGG AAC Leu Arg Val Leu Ile Trp Phe Ile Ser Ile Leu Ala Ile Thr Gly Asn 365 370 375 380	1214
ATC ATA GTG CTA GTG ATC CTA ACT ACC AGC CAA TAT AAA CTC ACA GTC Ile Ile Val Leu Val Ile Leu Thr Thr Ser Gln Tyr Lys Leu Thr Val 385 390 395	1262
CCC AGG TTC CTT ATG TGC AAC CTG GCC TTT GCT GAT CTC TGC ATT GGA Pro Arg Phe Leu Met Cys Asn Leu Ala Phe Ala Asp Leu Cys Ile Gly 400 405 410	1310
ATC TAC CTG CTG CTC ATT GCA TCA GTT GAT ATC CAT ACC AAG AGC CAA Ile Tyr Leu Leu Ile Ala Ser Val Asp Ile His Thr Lys Ser Gln 415 420 425	1358
TAT CAC AAC TAT GCC ATT GAC TGG CAA ACT GGG GCA GGC TGT GAT GCT Tyr His Asn Tyr Ala Ile Asp Trp Gln Thr Gly Ala Gly Cys Asp Ala 430 435 440	1406
GCT GGC TTT TTC ACT GTC TTT GCC AGT GAG CTG TCA GTC TAC ACT CTG Ala Gly Phe Phe Thr Val Phe Ala Ser Glu Leu Ser Val Tyr Thr Leu 445 450 455 460	1454
ACA GCT ATC ACC TTG GAA AGA TGG CAT ACC ATC ACG CAT GCC ATG CAG Thr Ala Ile Thr Leu Glu Arg Trp His Thr Ile Thr His Ala Met Gln 465 470 475	1502
CTG GAC TGC AAG GTG CAG CTC CGC CAT GCT GCC AGT GTC ATG GTG ATG Leu Asp Cys Lys Val Gln Leu Arg His Ala Ala Ser Val Met Val Met 480 485 490	1550
GGC TGG ATT TTT GCT TTT GCA GCT GCC CTC TTT CCC ATC TTT GGC ATC Gly Trp Ile Phe Ala Phe Ala Ala Leu Phe Pro Ile Phe Gly Ile 495 500 505	1598

- 21 -

AGC AGC TAC ATG AAG GTG AGC ATC TGC CTG CCC ATG GAT ATT GAC AGC Ser Ser Tyr Met Lys Val Ser Ile Cys Leu Pro Met Asp Ile Asp Ser 510 515 520	1646
CCT TTG TCA CAG CTG TAT GTC ATG TCC CTC CTT GTG CTC AAT GTC CTG Pro Leu Ser Gln Leu Tyr Val Met Ser Leu Leu Val Leu Asn Val Leu 525 530 535 540	1694
GCC TTT GTG GTC ATC TGT GGC TGC TAT ATC CAC ATC TAC CTC ACA GTG Ala Phe Val Val Ile Cys Gly Cys Tyr Ile His Ile Tyr Leu Thr Val 545 550 555	1742
CGG AAC CCC AAC ATC GTG TCC TCC TCT AGT GAC ACC AGG ATC GCC AAG Arg Asn Pro Asn Ile Val Ser Ser Ser Asp Thr Arg Ile Ala Lys 560 565 570	1790
CGC ATG GCC ATG CTC ATC TTC ACT GAC TTC CTC TGC ATG GCA CCC ATT Arg Met Ala Met Leu Ile Phe Thr Asp Phe Leu Cys Met Ala Pro Ile 575 580 585	1838
TCT TTC TTT GCC ATT TCT GCC TCC CTC AAG GTG CCC CTC ATC ACT GTG Ser Phe Ala Ile Ser Ala Ser Leu Lys Val Pro Leu Ile Thr Val 590 595 600	1886
TCC AAA GCA AAG ATT CTG CTG GTT CTG TTT CAC CCC ATC AAC TCC TGT Ser Lys Ala Lys Ile Leu Leu Val Leu Phe His Pro Ile Asn Ser Cys 605 610 615 620	1934
GCC AAC CCC TTC CTC TAT GCC ATC TTT ACC AAA AAC TTT CGC AGA GAT Ala Asn Pro Phe Leu Tyr Ala Ile Phe Thr Lys Asn Phe Arg Arg Asp 625 630 635	1982
TTC TTC ATT CTG CTG AGC AAG TGT GGC TGC TAT GAA ATG CAA GCC CAA Phe Phe Ile Leu Leu Ser Lys Cys Gly Cys Tyr Glu Met Gln Ala Gln 640 645 650	2030
ATT TAT AGG ACA GAA ACT TCA TCC ACT GTC CAC AAC ACC CAT CCA AGG Ile Tyr Arg Thr Glu Thr Ser Ser Thr Val His Asn Thr His Pro Arg 655 660 665	2078
AAT GGC CAC TGC TCT TCA GCT CCC AGA GTC ACC AAT GGT TCC ACT TAC Asn Gly His Cys Ser Ser Ala Pro Arg Val Thr Asn Gly Ser Thr Tyr 670 675 680	2126
ATA CTT GTC CCT CTA AGT CAT TTA GCC CAA AAC TAAAAACACAA TGTGAAAATG Ile Leu Val Pro Leu Ser His Leu Ala Gln Asn 685 690 695	2179

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 62 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGCCCCGCCGC GCCCCGCGCC CGGGCCGCCG CCCCCGCCG GACTTATGCA ATGAAGTGGT	60
TG	62

- 22 -

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTGAAAAAGC CAGCAGCATC

20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGCCCGCCGC GCCCGCGCC CGGCCCGCCG CCCCGCCCG ATTGACTGGC AAACTGGGG

59

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGAGGAGGAC ACGATGTTGG

20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 62 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGCCCGCCGC GCCCGCGCC CGGCCCGCCG CCCCGCCCG GGCTGCTATA TCCACATCTA

60

CC

62

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 23 -

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGAACCGAGC AGAATCTTTG C

21

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCCCCGCCGC GCCCCCGGCC CGGGCCCGCCG CCCCCGCCCG CTTTCTTTGC CATTCTGCC

60

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAAAGGCAAG GACTGAATTA TC

22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGCAAAGATT CTGCTGGTTC

20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TAGTTTTGGG CTAAATGACT TAGAGGG

27

- 24 -

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCTGCTCCTG GTCTCTTG TG

22

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGAAATTCTT TCGTGGGGCT

20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTTTGCAAAG GCACAGCAAT

20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTTATTTCAAG ATGGCTGAAT AAG

23

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- 25 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCTCATCTAG TTGGGTTC

18

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GAGTGCACCA TGGCGGTGT GAAAT

25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGGATTCAAG AAATAACACAA CTGTGTATTG AATGGAACCC

40

- 26 -

CLAIMS

We claim:

1. A method for determining a follicle-stimulating hormone receptor (*fshr*) genotype in a human patient, comprising the steps of:
 - 5 (a) providing a biological sample comprising nucleic acid from said patient, said nucleic acid including said patient's *fshr* alleles;
 - (b) analyzing said nucleic acid for the presence of a mutation or mutations in codon 189 of said *fshr* alleles; and
 - (c) determining an *fshr* genotype from said analyzing step, wherein the presence of a mutation in codon 189 of a *fshr* allele is correlated with an ovarian dysgenesis genotype.
- 10 2. The method according to claim 1 wherein said biological sample is a cell sample.
- 15 3. The method according to claim 1 or 2 wherein said patient is a female.
4. The method according to any one of claims 1-3 wherein said analyzing comprises sequencing a portion of said nucleic acid, said portion comprising codon 189 of said *fshr* alleles.
- 20 5. The method according to any one of claims 1-4 wherein said nucleic acid is DNA.
6. The method according to claim 5 wherein said analyzing step comprises the steps of:
 - 25 (a) exposing said nucleic acid to a restriction endonuclease having a recognition site that includes codon 189 of a wild type *fshr* allele, under conditions wherein said restriction endonuclease specifically cleaves DNA at its recognition site; and

- 27 -

(b) detecting said nucleic acid or polynucleotide restriction fragments thereof from said exposing step.

7. The method according to claim 6 wherein said restriction endonuclease is selected from the group consisting of *BsmI*, restriction endonucleases recognizing sites that overlap a *BsmI* site, restriction endonucleases recognizing the same cleavage sites as *BsmI*, and isoschizomers of *BsmI*.

8. The method according to claim 6 wherein said restriction endonuclease is *BsmI*.

9. A method for screening for an ovarian dysgenesis genotype in a patient, comprising the steps of:

(a) providing a biological sample comprising nucleic acid from said patient, said nucleic acid including said patient's follicle-stimulating hormone receptor (*fshr*) alleles;

(b) amplifying a portion of said nucleic acid to generate amplified DNA, said portion including codon 189 of said *fshr* alleles;

(c) exposing said amplified DNA to a restriction endonuclease selected from the group consisting of *BsmI* restriction endonuclease and restriction endonucleases having a recognition site which overlaps a *BsmI* recognition site, under conditions wherein said restriction endonuclease specifically cleaves DNA at the recognition site of said restriction endonuclease;

(d) thereafter detecting said amplified DNA or restriction fragments thereof;

(e) comparing the amplified DNA or fragments thereof of step (d) to control nucleic acid of a human subject free of an ovarian dysgenesis genotype, wherein said control nucleic acid has been amplified, exposed, and detected in accordance with steps (b), (c), and (d); and

(f) screening for an ovarian dysgenesis genotype from said comparison, wherein an ovarian dysgenesis genotype in said patient is correlated with a

- 28 -

different number of detected amplified DNA or restriction fragments thereof from said patient's amplified DNA than from said control amplified DNA.

10. The method according to claim 9 wherein in said screening step a homozygous ovarian dysgenesis genotype is correlated with fewer detected restriction fragments from said patient's amplified DNA than from said control amplified DNA.

11. The method according to claim 9 wherein in said screening step a heterozygous ovarian dysgenesis genotype is correlated with a greater number of detected amplified DNA or restriction fragments thereof from said patient's amplified DNA than from said control amplified DNA.

12. The method according to claim 9 wherein said patient is a human female.

13. The method according to claim 9 wherein said portion comprises exon 7 of said *fshr* alleles.

15 14. A method for diagnosing ovarian dysgenesis in a female patient, comprising the steps of:

obtaining a cell sample from a female patient;
isolating nucleic acids from said cell sample;
amplifying a portion of said nucleic acids encoding a receptor for
20 follicle-stimulating hormone, thereby generating amplified DNA;
exposing said amplified DNA to a restriction endonuclease selected
from the group consisting of *Bsm*I and restriction endonucleases having a recogni-
tion site which overlaps that of *Bsm*I, under conditions wherein said restriction
endonuclease specifically cleaves DNA at its recognition site;
25 detecting polynucleotide restriction fragments of said amplified
DNA; and

- 29 -

diagnosing ovarian dysgenesis from said restriction fragments, wherein ovarian dysgenesis is correlated with a C to T mutation in codon 189 of said DNA encoding a receptor for follicle-stimulating hormone, said mutation eliminating a recognition site of said restriction endonuclease.

5 15. A method for diagnosing ovarian dysgenesis in a female patient comprising the steps of:

obtaining a cell sample from a female patient;
isolating nucleic acids from said cell sample;
amplifying a portion of said nucleic acids comprising exon 7 of a
10 follicle-stimulating hormone receptor gene, said portion excluding any *BsmI* site outside of exon 7, thereby generating amplified DNA;
exposing said amplified DNA to a restriction endonuclease selected from the group consisting of *BsmI* and restriction endonucleases having a recognition site which overlaps that of *BsmI*, under conditions wherein said restriction
15 endonuclease specifically cleaves DNA at its recognition site;
detecting said amplified DNA or polynucleotide restriction fragments thereof after said exposing step; and
diagnosing ovarian dysgenesis from said detecting step, wherein
ovarian dysgenesis is correlated with the detection of said amplified DNA and the
20 absence of polynucleotide restriction fragments thereof.

16. The method according to claim 14 or 15, wherein said restriction endonucleases having a recognition site which overlaps that of *BsmI* are selected from the group consisting of *SacIII* and *SstIII*.

25 17. A method for diagnosing ovarian dysgenesis in a female patient suspected of having ovarian dysgenesis, comprising the steps of:
obtaining a cell sample from said female patient;
isolating nucleic acid from said cell sample;

- 30 -

amplifying a portion of said isolated nucleic acid comprising exon 7
of a follicle-stimulating hormone receptor gene, thereby generating amplified DNA;
sequencing said amplified DNA;
comparing the sequence of said amplified DNA to SEQ ID NO:1;

5 and

diagnosing ovarian dysgenesis by the presence of one or more se-
quence differences between the sequence of said amplified DNA and SEQ ID
NO:1.

18. The method according to claim 17, wherein said one or more diffe-
10 rences between the sequence of said amplified DNA and SEQ ID NO:1 comprises
a difference at nucleotide position 640 in SEQ ID NO:1.

19. The method according to claim 18, wherein a cytidylate at position
640 in SEQ ID NO:1 is a thymidylate at a corresponding position in said amplified
DNA.

1/10

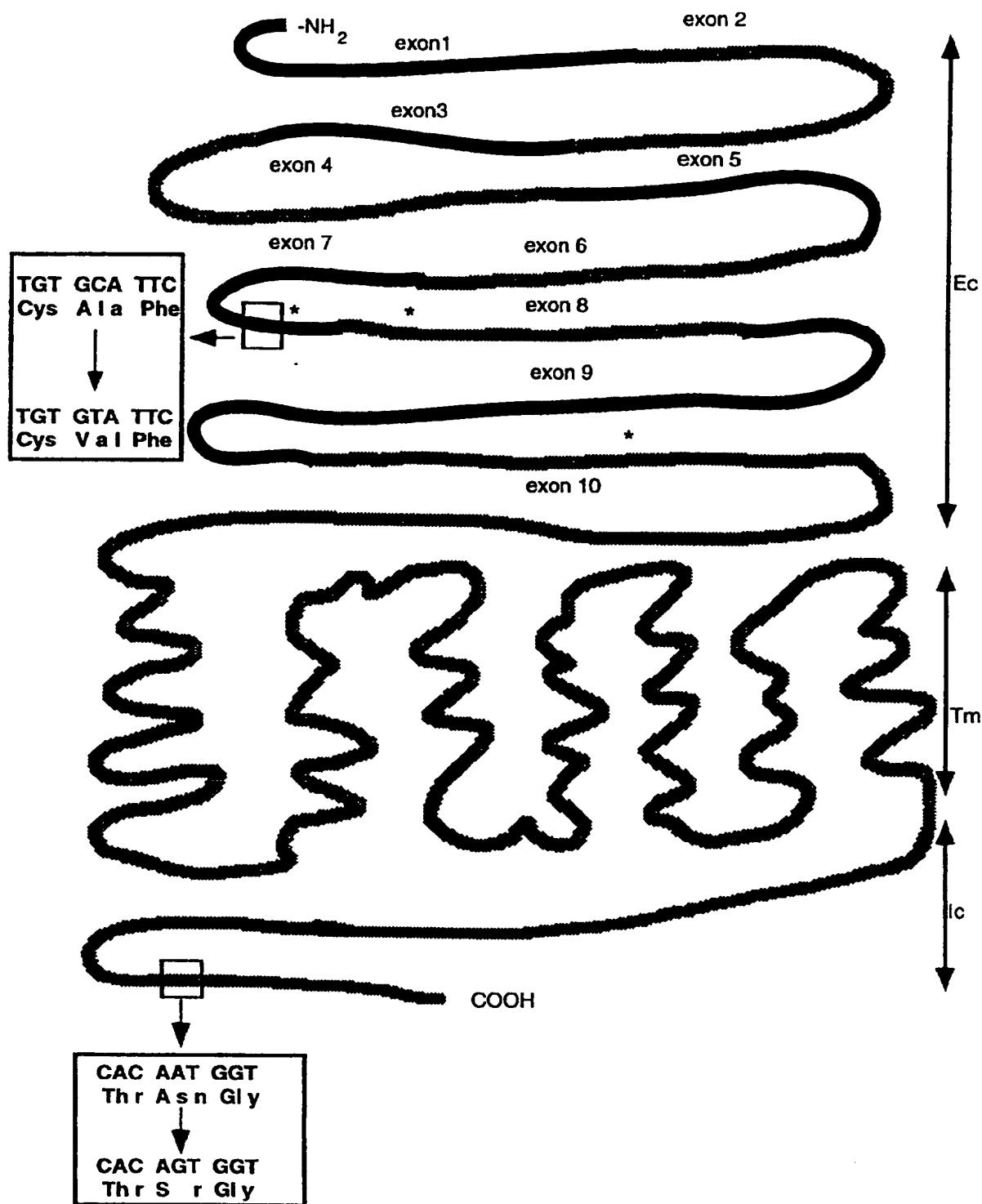


FIG. 1

2/10

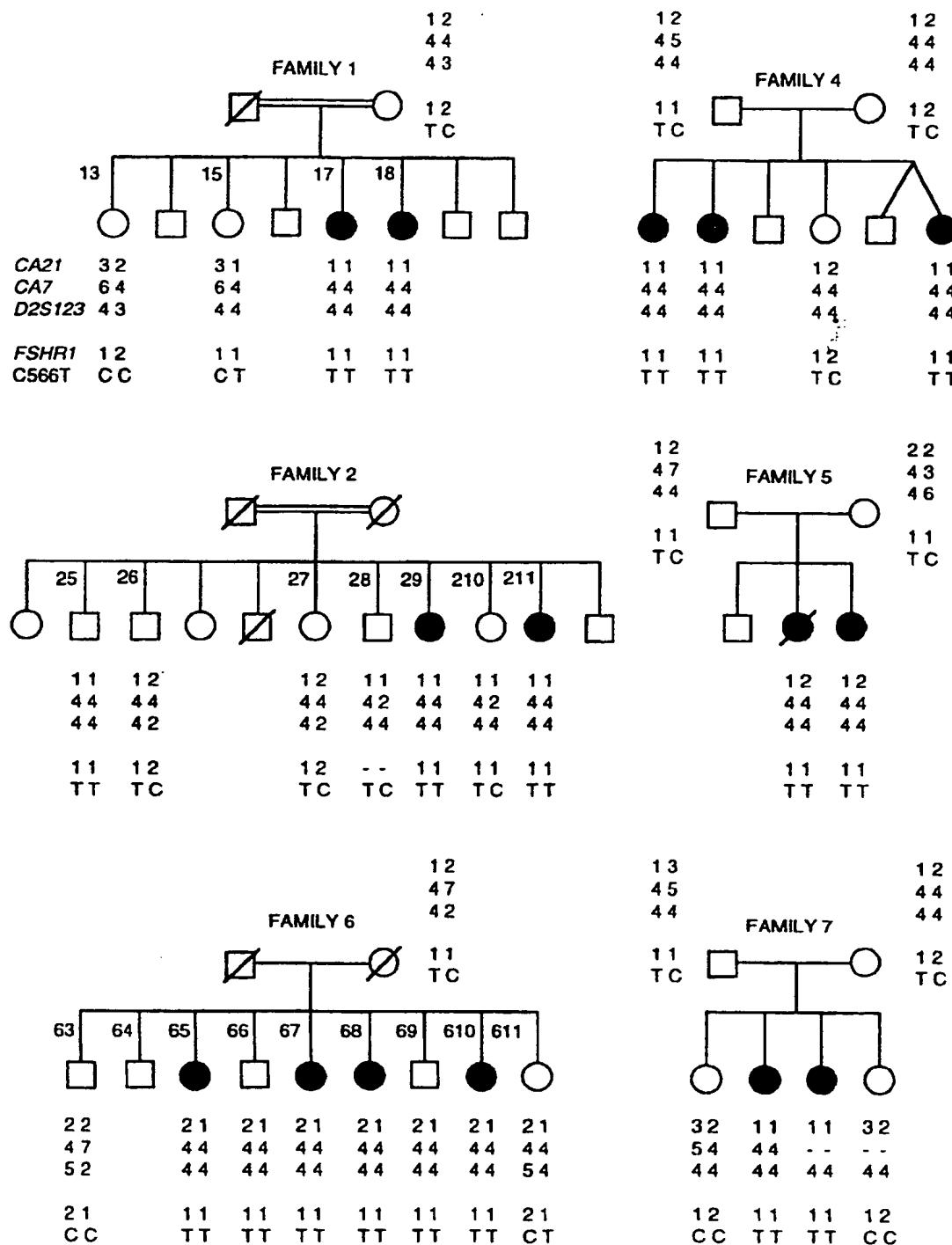


FIG. 2

3/10

PTBAB 5-7 Py/D2S47

E28-T/D2S16

CRI L1247 M/D2S34

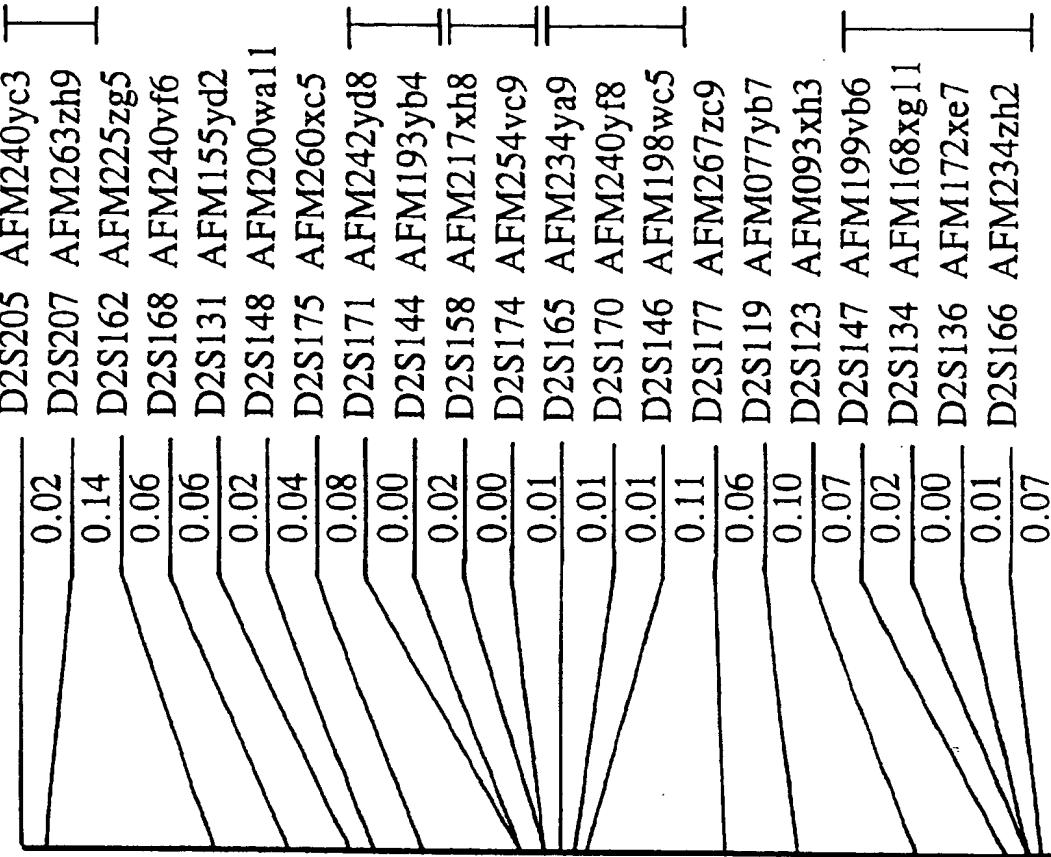


FIG. 3 (1 of 4)

4/10

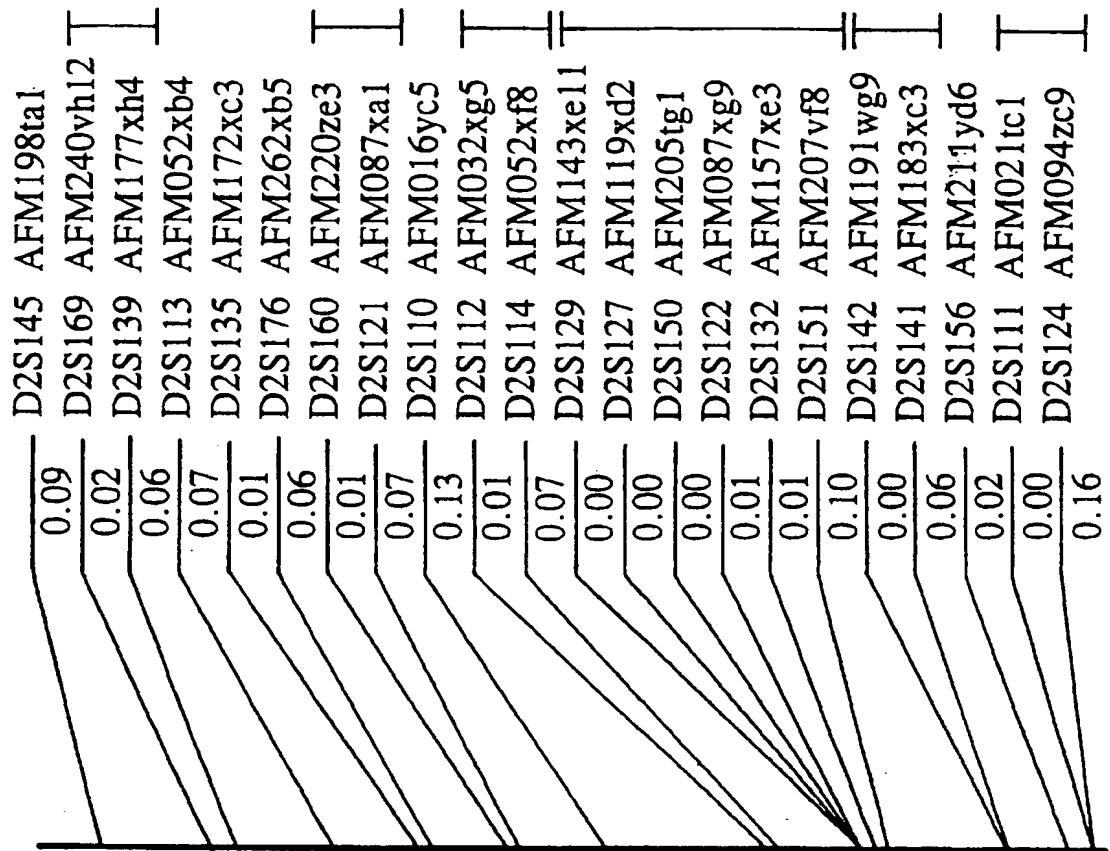


FIG. 3 (2 of 4)

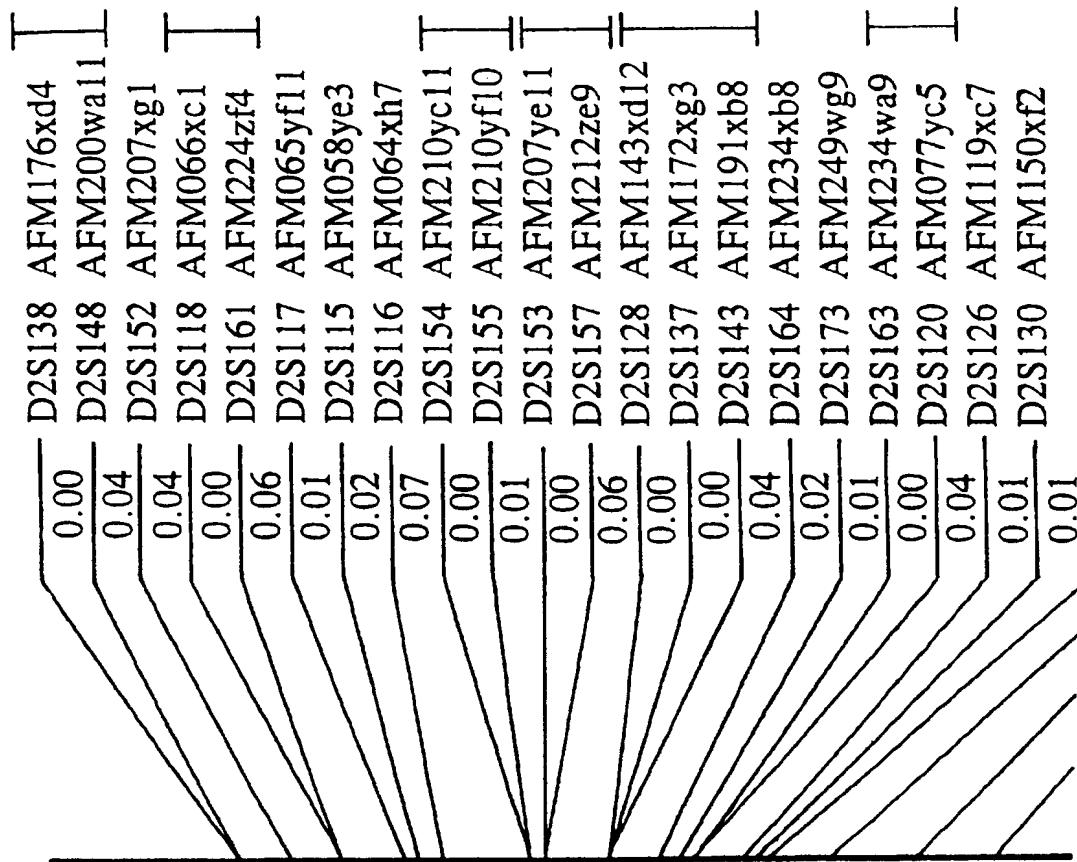
CRI C138 P/D2S25

CRI L34 M/D2S20

RECTIFIED SHEET (RULE 91)

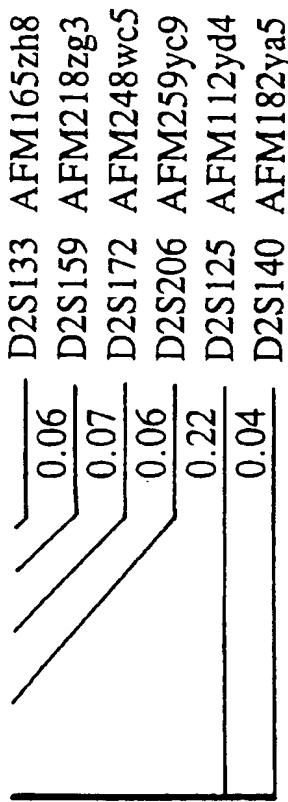
SEARCHED

5/10



6/10

FIG. 3 (4of 4)



7/10

TGTGGAGCTT	CTGAGATCTG	TGGAGGTTTT	TCTCTGCAAA	TGCAGGAAGA	AATCAGGTGG	60										
ATGGATGCAT	AATT	ATG	GCC	CTG	CTC	CTG	GTC	TCT	TTG	CTG	GCA	TTC	CTG	110		
		Met	Ala	Leu	Leu	Leu	Val	Ser	Leu	Leu	Ala	Phe	Leu	12		
AGC	TTG	GGC	TCA	GGA	TGT	CAT	CAT	CGG	ATC	TGT	CAC	TGC	TCT	AAC	AGG	158
Ser	Leu	Gly	Ser	Gly	Cys	His	His	Arg	Ile	Cys	His	Cys	Ser	Asn	Arg	28
GTT	TTT	CTC	TGC	CAA	GAG	AGC	AAG	GTG	ACA	GAG	ATT	CCT	TCT	GAC	CTC	206
Val	Phe	Leu	Cys	Gln	Glu	Ser	Lys	Val	Thr	Glu	Ile	Pro	Ser	Asp	Leu	44
CCG	AGG	AAT	GCC	ATT	GAA	CTG	AGG	TTT	GTC	CTC	ACC	AAG	CTT	CGA	GTC	254
Pro	Arg	Asn	Ala	Ile	Glu	Leu	Arg	Phe	Val	Leu	Thr	Lys	Leu	Arg	Val	60
ATC	CAA	AAA	GGT	GCA	TTT	TCA	GGA	TTT	GGG	GAC	CTG	GAG	AAA	ATA	GAG	302
Ile	Gln	Lys	Gly	Ala	Phe	Ser	Gly	Phe	Gly	Asp	Leu	Glu	Lys	Ile	Glu	76
ATC	TCT	CAG	AAT	GAT	GTC	TTG	GAG	GTG	ATA	GAG	GCA	GAT	GTG	TTC	TCC	350
Ile	Ser	Gln	Asn	Asp	Val	Leu	Glu	Val	Ile	Glu	Ala	Asp	Val	Phe	Ser	92
AAC	CTT	CCC	AAA	TTA	CAT	GAA	ATT	AGA	ATT	GAA	AAG	GCC	AAC	AAC	CTG	398
Asn	Leu	Pro	Lys	Leu	His	Glu	Ile	Arg	Ile	Glu	Lys	Ala	Asn	Asn	Leu	108
CTC	TAC	ATC	AAC	CCT	GAG	GCC	TTC	CAG	AAC	CTT	CCC	AAC	CTT	CAA	TAT	446
Leu	Tyr	Ile	Asn	Pro	Glu	Ala	Phe	Gln	Asn	Leu	Pro	Asn	Leu	Gln	Tyr	124
CTG	TTA	ATA	TCC	AAC	ACA	GGT	ATT	AAG	CAC	CTT	CCA	GAT	GTT	CAC	AAG	494
Leu	Leu	Ile	Ser	Asn	Thr	Gly	Ile	Lys	His	Leu	Pro	Asp	Val	His	Lys	140
ATT	CAT	TCT	CTC	CAA	AAA	GTT	TTA	CTT	GAC	ATT	CAA	GAT	AAC	ATA	AAC	542
Ile	His	Ser	Leu	Gln	Lys	Val	Leu	Leu	Asp	Ile	Gln	Asp	Asn	Ile	Asn	156
ATC	CAC	ACA	ATT	GAA	AGA	AAT	TCT	TTC	GTG	GGG	CTG	AGC	TTT	GAA	AGT	590
Ile	His	Thr	Ile	Glu	Arg	Asn	Ser	Phe	Val	Gly	Leu	Ser	Phe	Glu	Ser	172
GTG	ATT	CTA	TGG	CTG	AAT	AAG	AAT	GGG	ATT	CAA	GAA	ATA	CAC	AAC	TGT	638
Val	Ile	Leu	Trp	Leu	Asn	Lys	Asn	Gly	Ile	Gln	Glu	Ile	His	Asn	Cys	188
GCA	TTC	AAT	GGA	ACC	CAA	CTA	GAT	GAG	CTG	AAT	CTA	AGC	GAT	AAT	AAT	686
Ala	Phe	Asn	Gly	Thr	Gln	Leu	Asp	Glu	Leu	Asn	Leu	Ser	Asp	Asn	Asn	204
AAT	TTA	GAA	GAA	TTG	CCT	AAT	GAT	GTT	TTC	CAC	GGA	GCC	TCT	GGA	CCA	734
Asn	Leu	Glu	Glu	Leu	Pro	Asn	Asp	Val	Phe	His	Gly	Ala	Ser	Gly	Pro	220
GTC	ATT	CTA	GAT	ATT	TCA	AGA	ACA	AGG	ATC	CAT	TCC	CTG	CCT	AGC	TAT	782
Val	Ile	Leu	Asp	Ile	Ser	Arg	Thr	Arg	Ile	His	Ser	Leu	Pro	Ser	Tyr	236
GGC	TTA	GAA	AAT	CTT	AAG	AAG	CTG	AGG	GCC	AGG	TCG	ACT	TAC	AAC	TTA	830
Gly	Leu	Glu	Asn	Leu	Lys	Lys	Leu	Arg	Ala	Arg	Ser	Thr	Tyr	Asn	Leu	252
AAA	AAG	CTG	CCT	ACT	CTG	GAA	AAG	CTT	GTC	GCC	CTC	ATG	GAA	GCC	AGC	878
Lys	Lys	Leu	Pro	Thr	Leu	Glu	Lys	Leu	Val	Ala	Leu	Met	Glu	Ala	Ser	268
CTC	ACC	TAT	CCC	AGC	CAT	TGC	TGT	GCC	TTT	GCA	AAC	TGG	AGA	CGG	CAA	926
Leu	Thr	Tyr	Pro	Ser	His	Cys	Cys	Ala	Phe	Ala	Asn	Trp	Arg	Arg	Gln	284
ATC	TCT	GAG	CTT	CAT	CCA	ATT	TGC	AAC	AAA	TCT	ATT	TTA	AGG	CAA	GAA	974
Ile	Ser	Glu	Leu	His	Pro	Ile	Cys	Asn	Lys	Ser	Ile	Leu	Arg	Gln	Glu	300
GTT	GAT	TAT	ATG	ACT	CAG	ACT	AGG	GGT	CAG	AGA	TCC	TCT	CTG	GCA	GAA	1022
Val	Asp	Tyr	Met	Thr	Gln	Thr	Arg	Gly	Gln	Arg	Ser	Ser	Leu	Ala	Glu	316
GAC	AAT	GAG	TCC	AGC	TAC	AGC	AGA	GGA	TTT	GAC	ATG	ACG	TAC	ACT	GAG	1070
Asp	Asn	Glu	Ser	Ser	Tyr	Ser	Arg	Gly	Phe	Asp	Met	Thr	Tyr	Thr	Glu	332
TTT	GAC	TAT	GAC	TTA	TGC	AAT	GAA	GTG	GTT	GAC	GTG	ACC	TGC	TCC	CCT	1118
Phe	Asp	Tyr	Asp	Leu	Cys	Asn	Glu	Val	Val	Asp	Val	Thr	Cys	Ser	Pro	348

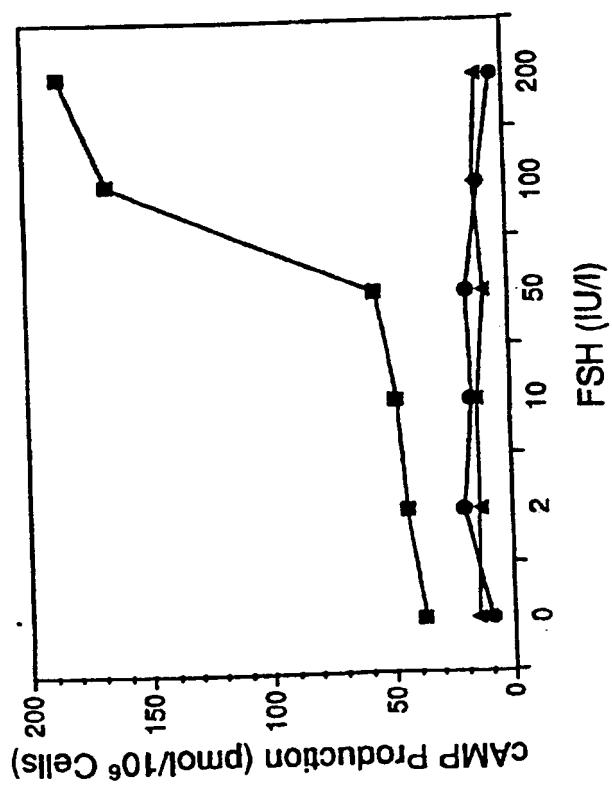
8/10

AAG CCA GAT GCA TTC AAC CCA TGT GAA GAT ATC ATG GGG TAC AAC ATC Lys Pro Asp Ala Phe Asn Pro Cys Glu Asp Ile Met Gly Tyr Asn Ile	1166 364
CTC AGA GTC CTG ATA TGG TTT ATC AGC ATC CTG GCC ATC ACT GGG AAC Leu Arg Val Leu Ile Trp Phe Ile Ser Ile Leu Ala Ile Thr Gly Asn	1214 380
ATC ATA GTG CTA GTG ATC CTA ACT ACC AGC CAA TAT AAA CTC ACA GTC Ile Ile Val Leu Val Ile Leu Thr Thr Ser Gln Tyr Lys Leu Thr Val	1262 396
CCC AGG TTC CTT ATG TGC AAC CTG GCC TTT GCT GAT CTC TGC ATT GGA Pro Arg Phe Leu Met Cys Asn Leu Ala Phe Ala Asp Leu Cys Ile Gly	1310 412
ATC TAC CTG CTG CTC ATT GCA TCA GTT GAT ATC CAT ACC AAG AGC CAA Ile Tyr Leu Leu Ile Ala Ser Val Asp Ile His Thr Lys Ser Gln	1358 428
TAT CAC AAC TAT GCC ATT GAC TGG CAA ACT GGG GCA GGC TGT GAT GCT Tyr His Asn Tyr Ala Ile Asp Trp Gln Thr Gly Ala Gly Cys Asp Ala	1406 444
GCT GGC TTT TTC ACT GTC TTT GCC AGT GAG CTG TCA GTC TAC ACT CTG Ala Gly Phe Phe Thr Val Phe Ala Ser Glu Leu Ser Val Tyr Thr Leu	1454 460
ACA GCT ATC ACC TTG GAA AGA TGG CAT ACC ATC ACG CAT GCC ATG CAG Thr Ala Ile Thr Leu Glu Arg Trp His Thr Ile Thr His Ala Met Gln	1502 476
CTG GAC TGC AAG GTG CAG CTC CGC CAT GCT GCC AGT GTC ATG GTG ATG Leu Asp Cys Lys Val Gln Leu Arg His Ala Ala Ser Val Met Val Met	1550 492
GGC TGG ATT TTT GCT TTT GCA GCT GCC CTC TTT CCC ATC TTT GGC ATC Gly Trp Ile Phe Ala Phe Ala Ala Leu Phe Pro Ile Phe Gly Ile	1598 508
AGC AGC TAC ATG AAG GTG AGC ATC TGC CTG CCC ATG GAT ATT GAC AGC Ser Ser Tyr Met Lys Val Ser Ile Cys Leu Pro Met Asp Ile Asp Ser	1646 524
CCT TTG TCA CAG CTG TAT GTC ATG TCC CTC CTT GTG CTC AAT GTC CTG Pro Leu Ser Gln Leu Tyr Val Met Ser Leu Leu Val Leu Asn Val Leu	1694 540
GCC TTT GTG GTC ATC TGT GGC TGC TAT ATC CAC ATC TAC CTC ACA GTG Ala Phe Val Val Ile Cys Gly Cys Tyr Ile His Ile Tyr Leu Thr Val	1742 556
CGG AAC CCC AAC ATC GTG TCC TCC TCT AGT GAC ACC AGG ATC GCC AAG Arg Asn Pro Asn Ile Val Ser Ser Asp Thr Arg Ile Ala Lys	1790 572
CGC ATG GCC ATG CTC ATC TTC ACT GAC TTC CTC TGC ATG GCA CCC ATT Arg Met Ala Met Leu Ile Phe Thr Asp Phe Leu Cys Met Ala Pro Ile	1838 588
TCT TTC TTT GCC ATT TCT GCC TCC CTC AAG GTG CCC CTC ATC ACT GTG Ser Phe Ala Ile Ser Ala Ser Leu Lys Val Pro Leu Ile Thr Val	1886 604
TCC AAA GCA AAG ATT CTG CTG GTT CTG TTT CAC CCC ATC AAC TCC TGT Ser Lys Ala Lys Ile Leu Leu Val Leu Phe His Pro Ile Asn Ser Cys	1934 620
GCC AAC CCC TTC CTC TAT GCC ATC TTT ACC AAA AAC TTT CGC AGA GAT Ala Asn Pro Phe Leu Tyr Ala Ile Phe Thr Lys Asn Phe Arg Arg Asp	1982 636
TTC TTC ATT CTG CTG AGC AAG TGT GGC TGC TAT GAA ATG CAA GCC CAA Phe Phe Ile Leu Leu Ser Lys Cys Gly Cys Tyr Glu Met Gln Ala Gln	2030 652
ATT TAT AGG ACA GAA ACT TCA TCC ACT GTC CAC AAC ACC CAT CCA AGG Ile Tyr Arg Thr Glu Thr Ser Ser Thr Val His Asn Thr His Pro Arg	2078 668
AAT GGC CAC TGC TCT TCA GCT CCC AGA GTC ACC AAT GGT TCC ACT TAC Asn Gly His Cys Ser Ser Ala Pro Arg Val Thr Asn Gly Ser Thr Tyr	2126 684
ATA CTT GTC CCT CTA AGT CAT TTA GCC CAA AAC TAAAAACACAA TGTGAAAATG Ile Leu Val Pro Leu Ser His Leu Ala Gln Asn	2179 695

FIG. 4 (2 of 2)

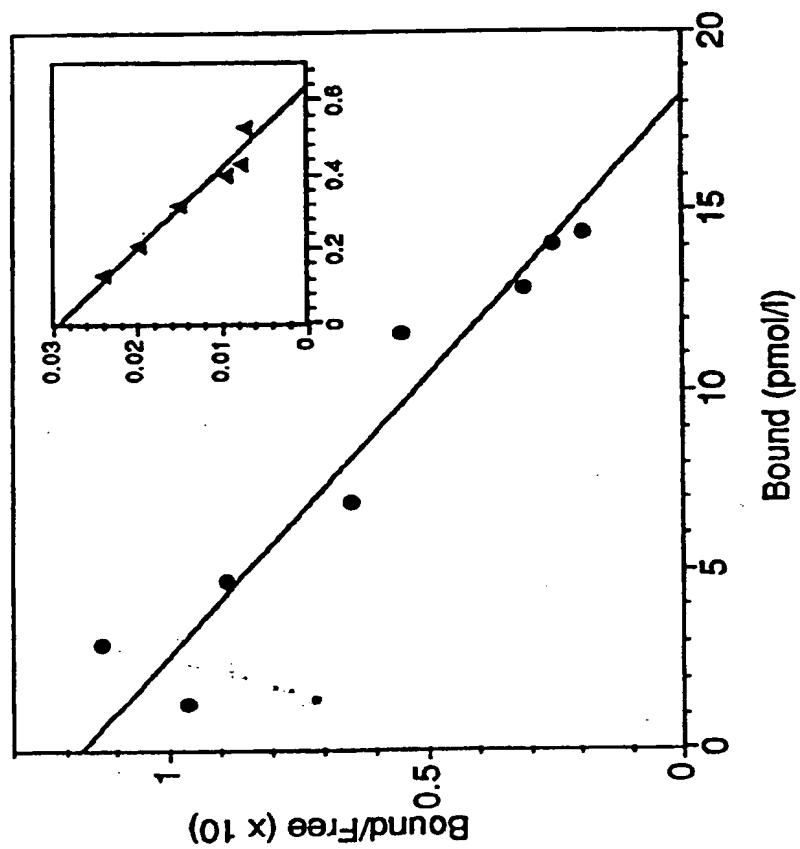
9/10

FIG. 5

**SUBSTITUTE SHEET (Rule 26)**

10/10

FIG. 6



INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/FI 96/00501

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12Q1/68 C07H21/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	<p>CELL, vol. 82, no. 6, 22 September 1995, pages 959-68, XP000609143 AITTOMÄKI, K. ET AL: "Mutation in the follicle-stimulating hormone receptor causes hereditary hypergonadotropic ovarian failure" see the whole document</p> <p>---</p>	1-18
A	<p>AMERICAN JOURNAL OF HUMAN GENETICS, vol. 54, no. 5, May 1994. pages 844-51, XP000612836 AITTOMÄKI, K.: "The genetics of XX gonadal dysgenesis" cited in the application see the whole document</p> <p>---</p> <p>-/-</p>	1

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *'A' document defining the general state of the art which is not considered to be of particular relevance
- *'E' earlier document but published on or after the international filing date
- *'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *'O' document referring to an oral disclosure, use, exhibition or other means
- *'P' document published prior to the international filing date but later than the priority date claimed

*'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

*'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

*'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

*'Z' document member of the same patent family

1

Date of the actual completion of the international search

11 December 1996

Date of mailing of the international search report

07.01.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Td. (+31-70) 340-2040, Tx. 31 651 epo nl.
 Fax: (+31-70) 340-3016

Authorized officer

Osborne, H

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/FI 96/00501

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NATURE GENETICS, vol. 9, February 1995, pages 160-4, XP000612866 KREMER H ET AL: "Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene" see the whole document</p> <p>---</p>	1
A	<p>GENOMICS , vol. 15, January 1993, pages 222-24, XP000612862 ROUSSEAU-MERCK M ET AL: "The chromosomal localization of the human follicle hormone receptor gene (FSHR) on 2p21-2p15 is similar to that of the luteinizing hormone receptor gene" cited in the application see the whole document</p> <p>-----</p>	1

